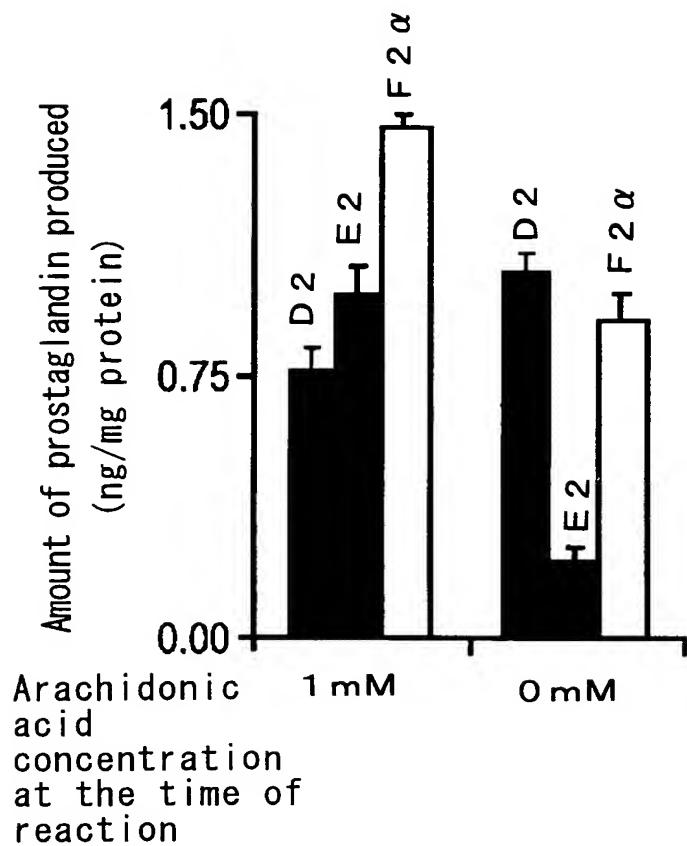
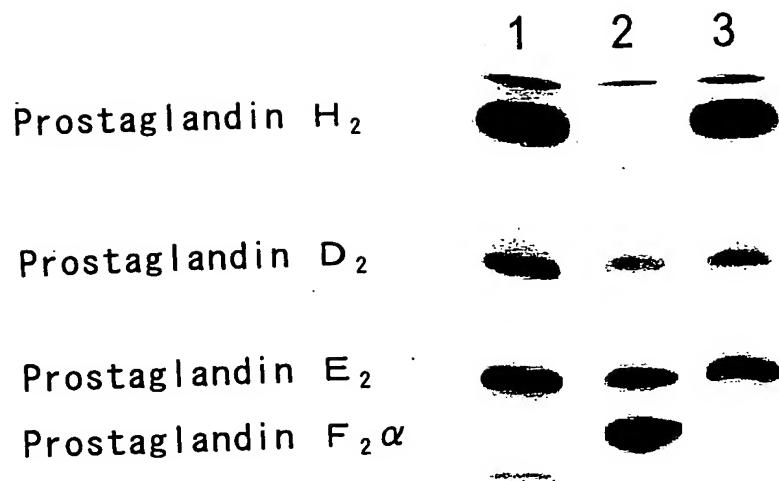


Fig. 1



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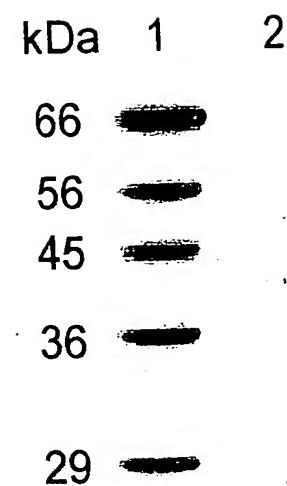
Fig. 2



1. No enzyme
2. *Trypanosoma cruzi* extract
3. *Trypanosoma cruzi* extract after heat treatment

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Fig. 3



1. Molecular-weight marker protein
2. Purified enzyme

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Fig. 4

Purification step	Total protein (mg)	Enzymatic activity (nmol/min)	Specific activity (nmol/min/mg protein)	Purification ratio
Soluble fraction	1 7 1 . 0	1 5 4	0 . 9	1 . 0
20-80% saturation ammonium sulfate fraction	1 2 7 . 0	1 5 0	1 . 2	1 . 3
Superdex 200	1 1 3 . 0	1 5 0	1 . 2	1 . 3
Ultrafiltration chromatogram	8 . 0	1 7 0	2 5 . 0	2 8 . 0
Hydrophobic chromatogram				
DEAE ion exchange chromatogram	2 . 8	1 8 0	6 4 . 0	7 1 . 0
Superdex 200 Ultrafiltration chromatogram 2nd time	0 . 3	2 1 0	7 0 0 . 0	7 7 8 . 0

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Fig. 5

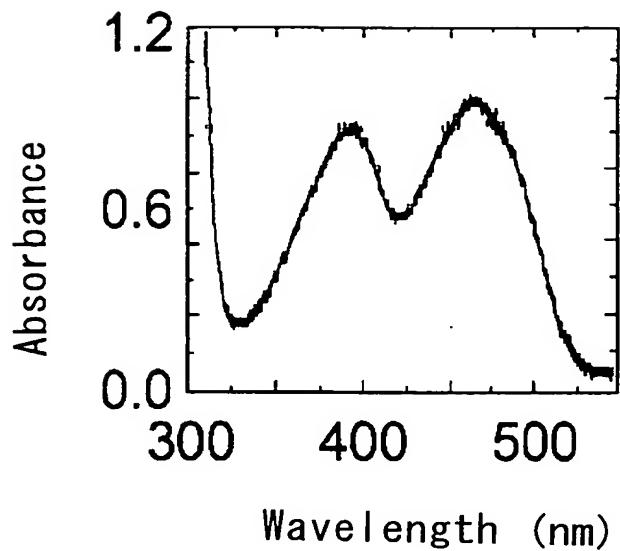
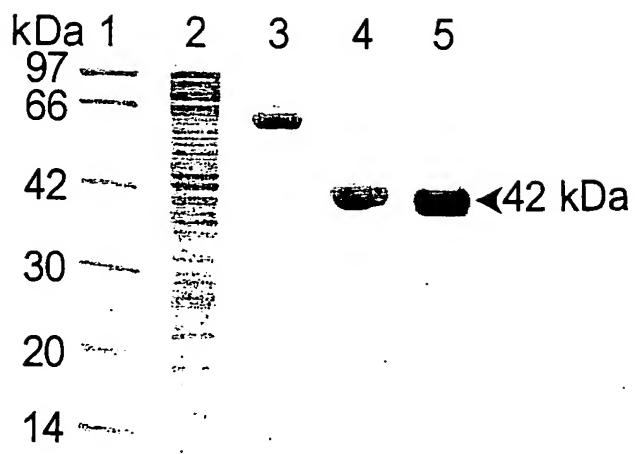


Fig. 6



1. Molecular weight marker protein
2. *E. coli* crude extract after transformation
3. Crude extract of *E. coli* expressing the recombinant TcOYE
4. Recombinant TcOYE collected by thrombin treatment
5. Purified standard of the recombinant TcOYE

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Fig. 7

## Substrate specificity of reduction by the recombinant TcOYE

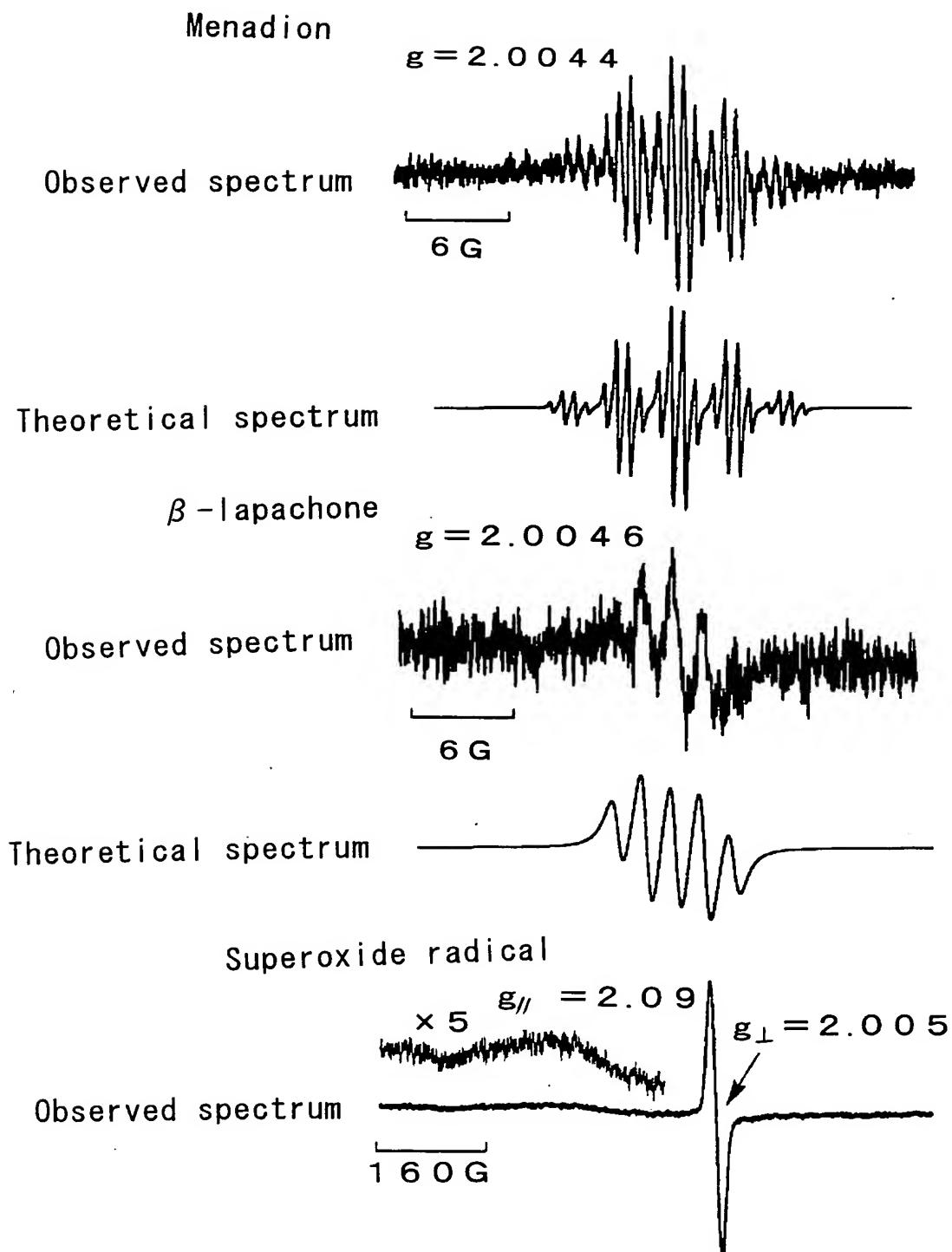
Substrate	Cofactor (10 $\mu$ M)	K <sub>m</sub> ( $\mu$ M)	V <sub>max</sub> /specific activity (nmol/min/mg)
9, 11-endoperoxide PGH <sub>2</sub>	NADH	—	554
	NADPH	5.0	766
Hydrogen peroxide BHP <sup>a</sup>	NADPH	2.3	99
	NADPH	n. d.	282
Menadione	NADH	—	499
	NADPH	0.82	700
$\beta$ -lapachone	NADH	0.17	650
	NADPH	—	433
4-nitroquinolin-N-oxide	NADH	—	759
	NADPH	9.5	1110
Nifurtimox	NADH	—	290
	NADPH	19.0	353
Phenazine methosulfate <sup>b</sup>	NADPH	10.4	235
Mevinolin <sup>c</sup>	NADH	n. d.	555
12-oxo-phytodienoic acid <sup>d</sup>	NADPH	n. d.	152
9-oxo ODE <sup>e</sup>	NADPH	n. d.	54
Econazole <sup>f</sup>	NADH	n. d.	43
Benznidazole	—	n. d.	N. D.
Miconazole <sup>g</sup>	—	n. d.	N. D.
Ketoconazole <sup>h</sup>	—	n. d.	N. D.
Crystal violet <sup>i</sup>	—	n. d.	N. D.
BHT <sup>j</sup>	—	n. d.	N. D.
BHA <sup>k</sup>	—	n. d.	N. D.

a: t-butyl hydroperoxide, b: 5-methyl-phenaziummethylsulfate), c: 2- $\beta$ , 6 $\alpha$ -dimethyl-8 $\alpha$ -(2-methyl-1-oxo-butoxy)-mevinic acid (lactone), d: 4-oxo-5 $\beta$ -(2Z-pentenyl)-2-cyclopentene-1 $\beta$ -octanoic acid), e: 9-oxo-10E, 12Z-octadecadienoic acid, f: 1-[2-([4-chlorophenyl] methoxy)-2-(2, 4-dichlorophenyl)ethyl-1H-imidazole], g: 1-[2, 4-dichloro  $\beta$ -([2, 4-dichlorobenzyl]-oxo)phenethyl] imidazole, h: cis-1-acetyl-4[4-[2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-yl-methyl)-1, 3-dioxolane-4-yl-methoxy]phenyl]piperazine], i: N-[4-[bis[4-(dimethylamino)-phenyl]methylene]-2, 5-cyclohexadiene-1-yl-iden-N-methyl-methane aluminum chloride, j: (2, 6di-tert-butyl-para-crezol), k: [2(3)-tert butyl-4-hydroxyanisole

N. D. : not detected

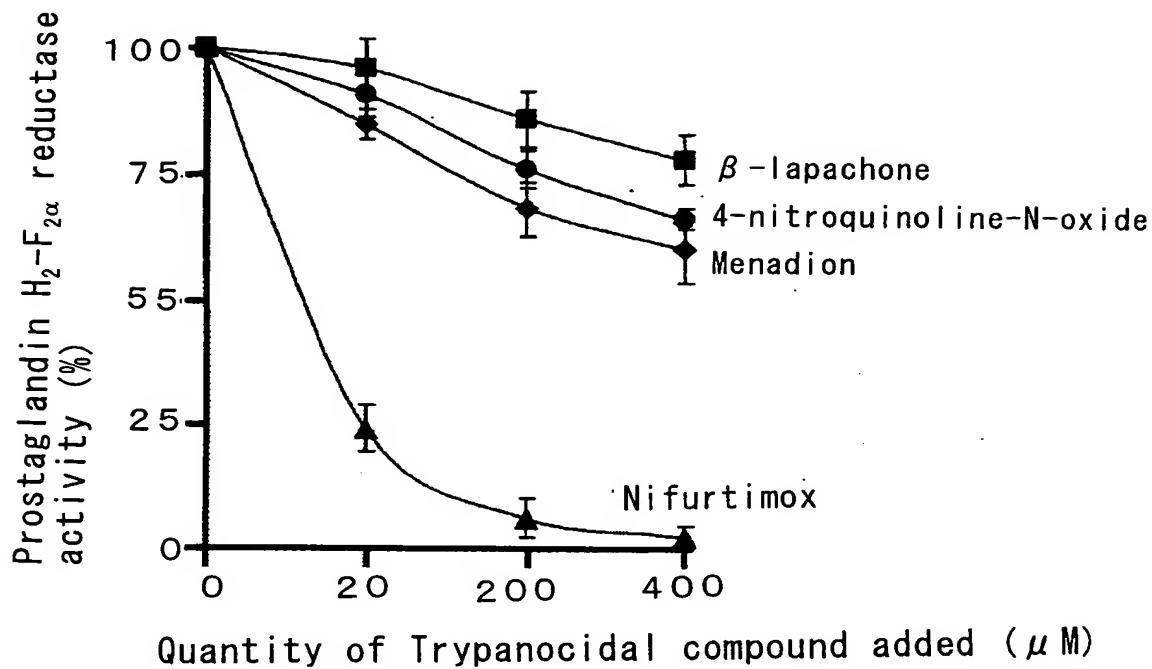
n. d. : not measured

Fig. 8



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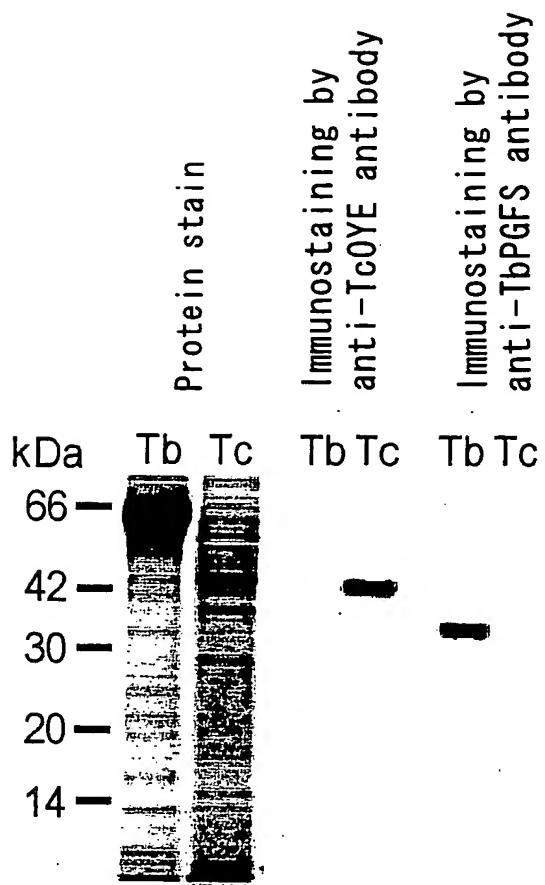
Fig. 9



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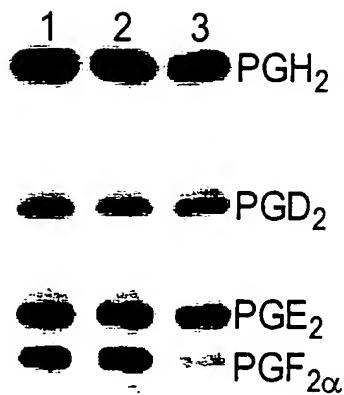
Fig. 10



Tb: Crude extract of *Trypanosoma brucei*

Tc: Crude extract of *Trypanosoma cruzi*

Fig. 11



1. Trypanosoma cruzi crude extract after reaction with control IgG
2. Trypanosoma cruzi crude extract after reaction with anti-TbPGFS antibody
3. Trypanosoma cruzi crude extract after reaction with anti-TcOYE antibody

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Fig. 12

Immunoprecipitation by the anti-Tc0YE antibody of the enzymatic activity to reduce menadion,  $\beta$ -lapachone, nifurtimox, 4-nitroquinoline-N-oxide in the crude extract of *Trypanosoma cruzi*

Sample	Persistent enzymatic activity (%)		
	Menadion	$\beta$ -lapachone	Nifurtimox
4-nitroquinoline-N-oxide			
Trypanosoma cruzi extract after reaction with anti-Tc0YE antibody	<sup>a</sup> N. D.	100(± 2)	N. D.
Trypanosoma cruzi extract after reaction with anti-TbPGFS antibody	98(± 8)	103(± 5)	100(± 10)
Trypanosoma cruzi extract after reaction with control bovine IgG	100(± 4)	100(± 10)	100(± 6)